14C leucine uptake in rat tissues at different times after irradiation.

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^{14}C LEUCINE UPTAKE IN RAT TISSUES AT DIFFERENT TIMES AFTER IRRADIATION

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Reports on protein synthesis are scarce and deal only with a few experimental points (LIPKIN & QUASTLER 1962, LIPKIN et coll. 1963, REUTER et coll. 1967).

The behaviour of tissue injured by previous irradiation at different times before killing of the animal has been evaluated in this experiment. ^{14}C leucine uptake in the TCA insoluble fraction has been used as a test.

The time of injection and killing were kept constant to avoid the influence of a circadian dependence although according to the present knowledge a different biologic effect of radiation cannot be excluded on the amino acid assumption when the exposure occurs at different hours of the day.

Material and Methods

Fifty-eight female Wistar rats 10 to 12 weeks old and weighing 160 to 170 g were kept at a light-darkness (L/D) cycle, 6.30 a.m. to 6.30 p.m., and fed with standard laboratory diet and water ad libitum.

In order to evaluate post-irradiation differences in the amino acid uptake each animal was injected intraperitoneally with 259 kBq (7 μCi) of L-leucine U^{14}C (Radiochemical Centre, Amersham, England; specific activity 370 MBq (10 mCi)/mmol, radiochemical purity 99%) in a 0.5 ml volume.

The injection was always given at 6.00 p.m. and 4 and 8 h later (at 10.00 p.m. and 2 a.m.) all rats were killed in 2 groups of 3 animals. Two groups of 5 animals were used as controls.

Forty-eight rats were whole-body irradiated with 8 Gy of γ rays from a telecobalt unit at 0, 4, 12, 20, 32, 44, 68, and 120 h before isotope injection.

Blood was taken by heart puncture. The small intestine was removed, longitudinally opened, gently washed in cold saline (0.9% NaCl) and then cut into 5 segments and homogenized at 10 per cent (w/v) with distilled water. Two small pieces, corresponding to the proximal and distal jejunum, were removed for microscopic and autoradiographic examinations. An Ilford K 5 emulsion was used. Kidneys were also removed and homogenized at 5 per cent.

The activity was assayed in the whole homogenate and in the supernatant after centrifugation at 900 g for 10 min. Homogenate and plasma were added with 2 parts of cold trichloroacetic acid (TCA) at 10 per cent and re-homogenized. After centrifugation the supernatant was used for TCA soluble activity assay. The pellet was washed twice with cold TCA, centrifuged, then dissolved with 0.1 N NaOH and counted for TCA insoluble activity.

The uptake of activity was expressed either as dpm/g protein or as total amount. This was done to

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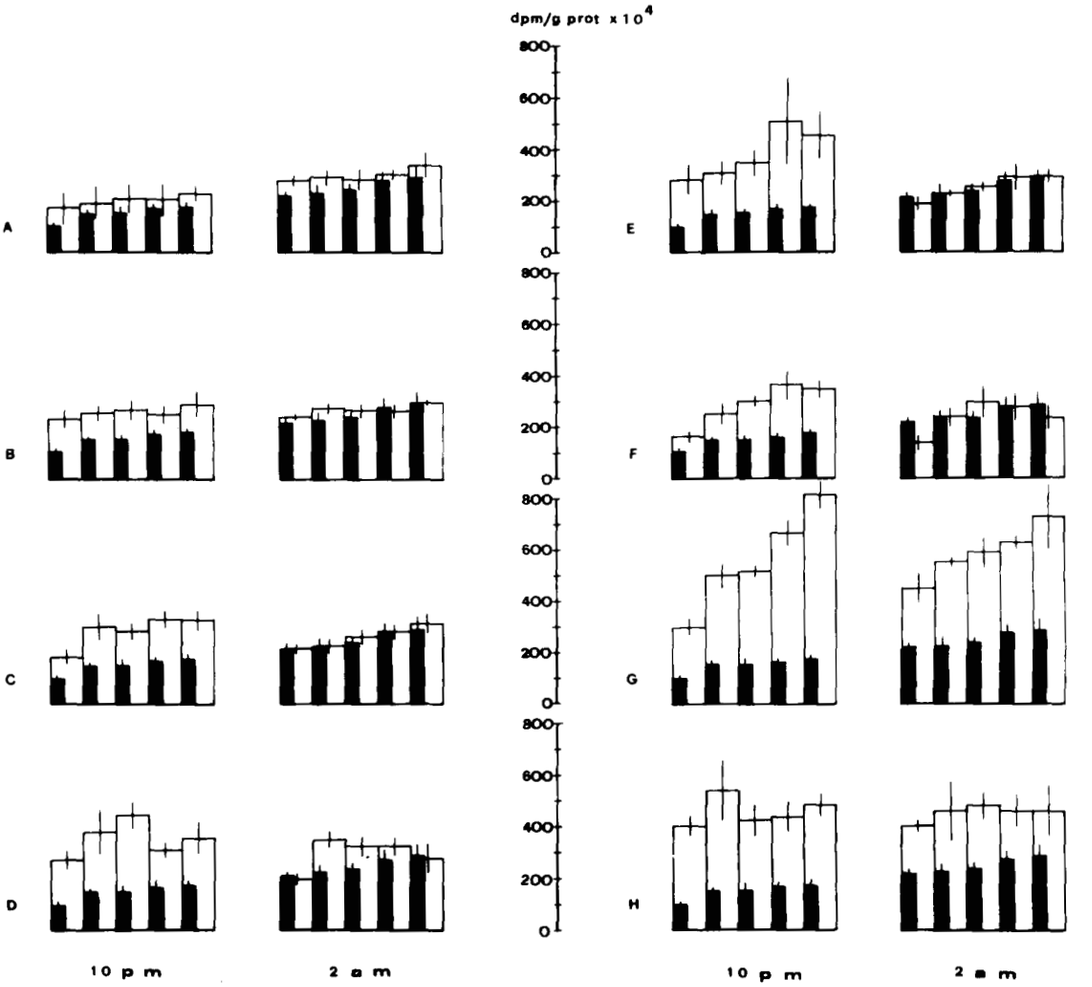

Fig. 1. Mean values ± SE of TCA insoluble activity as dpm/g protein at different intervals after irradiation (respectively from A to H: 0, 4, 12, 20, 32, 44, 68 and 120 h). Black columns represent control values.

take into account variations in body weight and particularly the weight of the small intestine, both varying during acute intestinal syndrome. Student's t-test was used to evaluate statistical significance of the differences.

Results

The TCA insoluble activity assayed in the centrifuged homogenate was always lower than in the whole homogenate, but no significant differences were observed. Therefore only the second result has been reported.

The data on the 5 segments of the small intestine in control and irradiated animals (Fig. 1) show that ^{14}C uptake increases from duodenum to terminal ileum. In controls the levels were higher at 8 than at 4 h after injection.

A significant increase in TCA insoluble activity was present in all irradiated groups killed at 10.00 p.m. except in the first; the highest values appeared in the 68 h interval. In the irradiated animals killed at 2.00 a.m. the TCA insoluble activity was significantly higher than in controls only in the groups killed at 68 and 120 h.

No significant differences in TCA soluble activity were observed between control groups (Fig. 2); in both cases the activity in the first tract was about twice as high as in the others. In some segments from irradiated animals killed at 10.00 p.m. an increase appeared in the groups between 12 and 68 h after irradiation. A reduction was observed in the

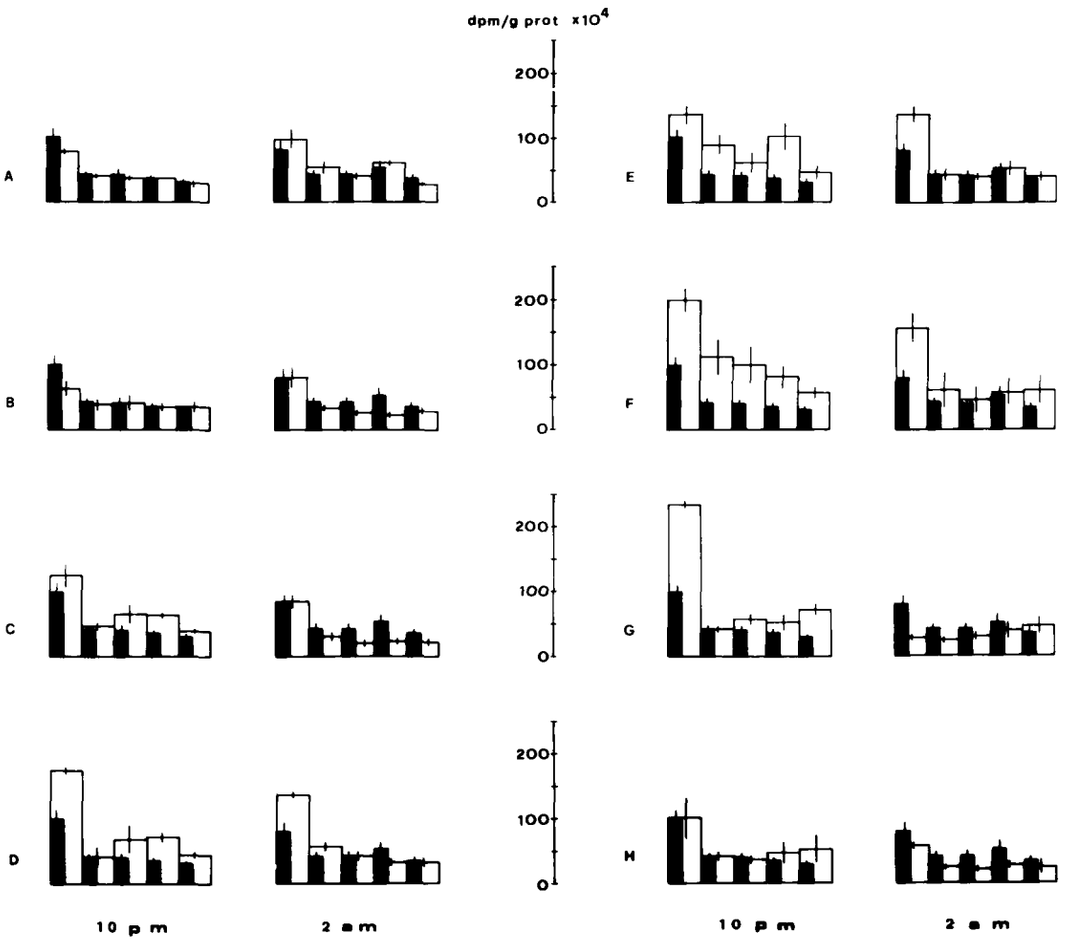

Fig. 2. Mean values ± SE of TCA soluble activity as dpm/g protein at different intervals after irradiation (respectively from A to H: 0, 4, 12, 20, 32, 44, 68 and 120 h). Black columns represent control values.

groups killed at 2.00 a.m. except for the first tract in which no reduction was present at 20, 32 and 44 h.

In Fig. 3 TCA insoluble and soluble activities are compared in the whole small intestine, kidneys and plasma. In the tissues from controls killed at 2.00 a.m. TCA insoluble activity was about twice as high as in the other group.

The behaviour of the whole small intestine in irradiated animals was similar to that reported for single segments. The activity was significantly higher in the animals killed at 10.00 p.m. while in the other groups an increase was observed only at 68 and 120 h.

In the kidneys and plasma of irradiated animals, killed at 10.00 p.m., TCA insoluble activity was always more than twice as high as in controls and even in the groups killed at 2.00 a.m. the levels were significantly higher. TCA soluble activity significantly increased in the kidneys of animals killed at 10.00 p.m. whereas only mild fluctuations were observed in the other groups. Plasma TCA soluble activity appeared higher than in controls in all irradiated groups, particularly when the insoluble fraction reached the highest levels.

The activity is reported as dpm/100 g body weight/small intestine weight, in Fig. 4 as per cent of controls in order to take into account modifications in body weight (marked reduction starting at 48 h) and small intestine weight (reduced as early as 20 h after irradiation and down to 65% of controls at 48 h) in irradiated animals.

The TCA insoluble activity showed values significantly higher than controls in the irradiated groups killed at 10.00 p.m. except at 32 and 44 h,

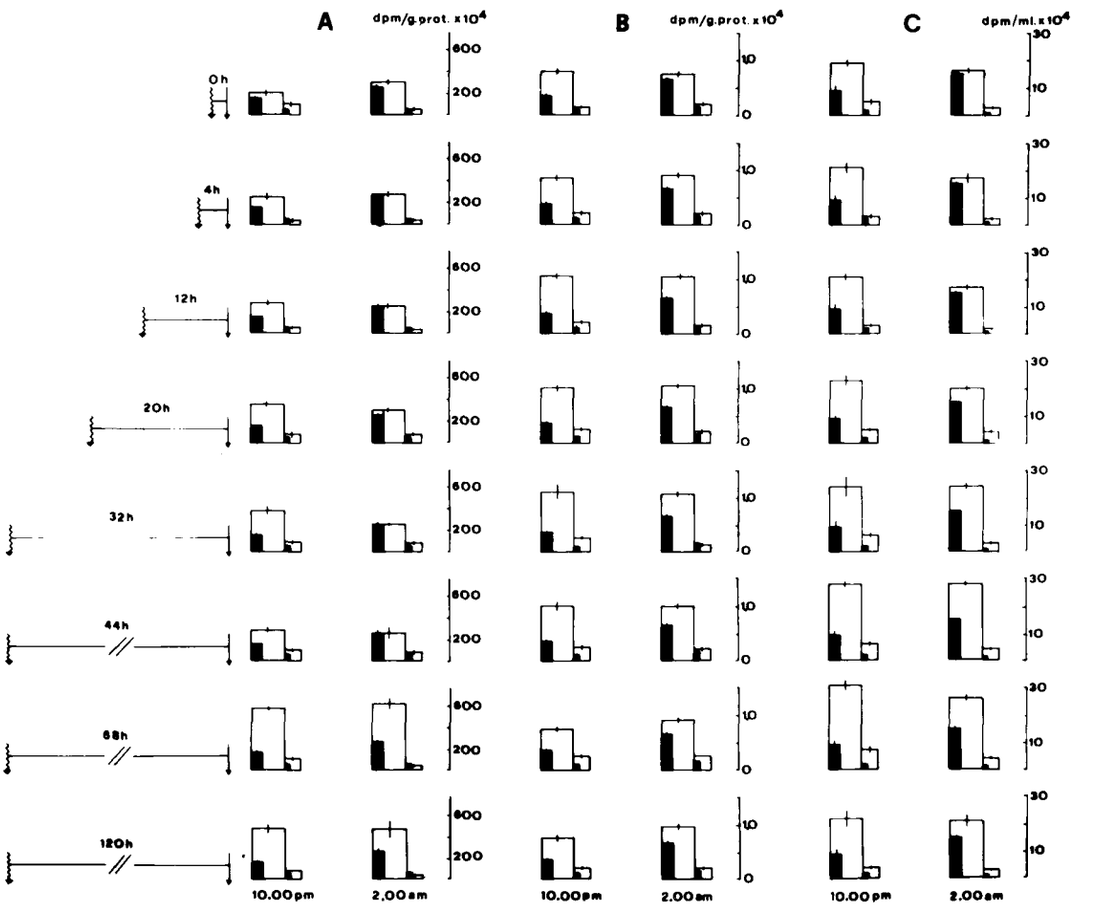

Fig. 3. Mean values ± SE of TCA insoluble (large column) and TCA soluble (small column) activity in the whole small intestine (A), kidney (B) and plasma (C) in the groups of animals killed at 10 p.m. and 2 a.m. Black column represents controls. ⦚ = Irradiation, ↓ = injection.

when a significant decrease was present. The values at 120 h reached 250 per cent of controls. In the animals killed at 2.00 a.m. a progressive reduction occurred reaching values of about 35 per cent in the 44 h group. At the next intervals control levels were reached and exceeded.

The TCA soluble activity in the 10.00 p.m. groups showed a reduction significant only at 32 h. In the groups killed at 2.00 a.m. the decrease was significant at 68 and 120 h when the TCA insoluble activity increased.

Discussion

Previously, CASATI et coll. (1979) demonstrated that retention of labelled protein in controls and rats irradiated with 8 Gy 2 h before injection showed a different behaviour between tissues with different proliferative activity and protein synthesis. In the small intestine, active protein synthesis and high turnover were confirmed by the high ^{14}C leucine uptake and by the rapid elimination of the tracer. After irradiation the maximum uptake occurred earlier and the elimination was more marked. Early appearance of the highest uptake was observed also in irradiated parotid glands, a tissue with low proliferation but high protein turnover, whereas the elimination curve overlapped controls (CREMONINI et coll. 1979). No significant differences between controls and irradiated animals appeared in skin and kidney (CASATI et coll.).

The present results show that an increased uptake of amino acid (expressed as dpm/g of protein or dpm/ml for plasma) appears at all intervals after irradiation. The increase was observed in all groups of animals killed 4 h after injection of ^{14}C leucine. In

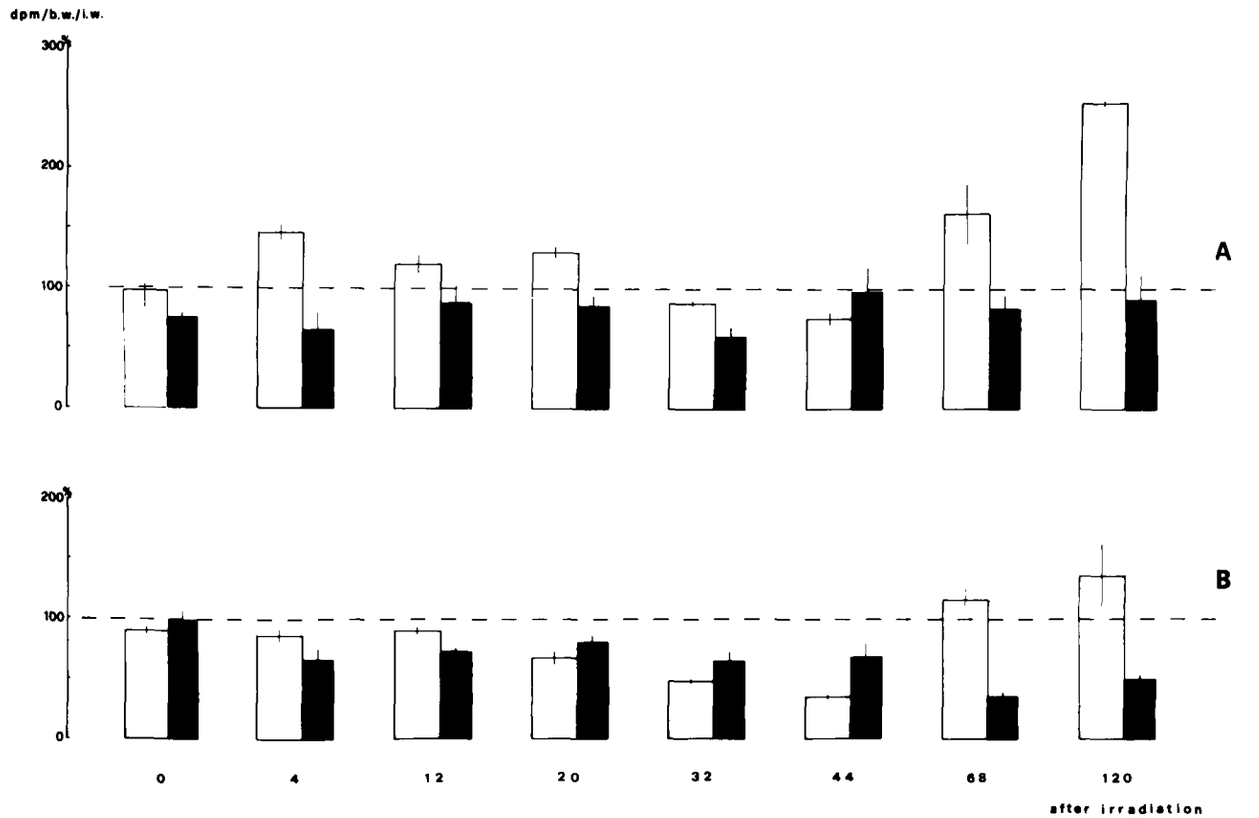

Fig. 4. Activity at different times (in hours) after irradiation as total dpm/100 g of body weight/small intestine weight; expressed as per cent of control values (dogged line). White column represents the activity in the whole homogenate and dark column the activity in TCA soluble fraction. A = rats killed at 10.00 p.m., B = rats killed at 2.00 a.m.

rats killed after 8 h the levels are similar to controls except for the small intestine in the 68 and 120 h groups, when the recovery phase is particularly evident. This confirms the faster eliminations of tracer from the TCA insoluble fraction.

The TCA soluble fraction does not increase in the same way as the TCA insoluble one; this accounts for the increased uptake of leucine in the protein fraction of the irradiated tissue. The TCA soluble fraction in the small intestine presents an evident increase only at the intervals when the crypts are more injured.

Also when activity is expressed as dpm/body weight/small intestine weight, the amount of uptake appears significantly higher in the animals killed at 10.00 p.m., in the groups where 4, 12 and 20 h elapsed between irradiation and injection. Previously, BECCIOLINI et coll. (1976) demonstrated that at these intervals a significant increase of brush border enzyme activities occurs. Reduced activity levels are present at 32 and 44 h when morphologic injury is more evident, whereas the maximum uptake is reached in the 120 h group. The TCA insoluble activity in the animals killed at 2.00 a.m. is generally significantly lower than controls and exceeds them only during the recovery phase.

It clearly appears from the results that irradiation modifies protein synthesis in tissues with different proliferative activity. The amount of the tracer in the small intestine depends on the time elapsed between irradiation and injection since it is somewhat correlated with the contemporary morphologic modifications. In fact autoradiographic observations demonstrate that at early intervals the increase of activity (spread in all cell structures) appears when morphologic injury is confined to the proliferative compartment. These results seem to confirm the previous hypothesis of an enhanced protein synthesis being the cause of the increase of brush border enzyme activities during this phase after irradiation (BECCIOLINI et coll. 1974, 1976).

At later intervals the lower total amount of tracer

appears when the epithelium progressively consists of cells reduced in number and with deep morphologic alterations, until the whole structure is involved. Cellular recovery is evident in the crypts of 68 h groups and corresponds to a marked increase of uptake. During this phase the increase might be due to a protein synthesis directed towards molecules of different biologic meaning. Morphologic data as well as the modifications of brush border enzymes (BECCIOLINI et coll. 1974) show that in the 68 h group an almost exclusive protein synthesis exists in the proliferative cells of the regenerating crypts. Both structural and functional proteins are synthetized at 120 h when the epithelium is formed by cells with a regular morphology, although enzyme activities, characterizing the differentiated cells, do not yet reach control levels.

SUMMARY

The uptake of ^{14}C leucine administered at different intervals after irradiation, but always 4 and 8 h before the animals were killed, has been evaluated in tissues with different proliferative activity and protein synthesis. The results have demonstrated an increased uptake and a more rapid elimination of the tracer after irradiation. In the small intestine a lower amount of TCA insoluble fraction was observed when the morphologic injury was evident, while protein synthesis significantly increased during the initial phase of appearance of the injury and mainly during the recovery phase of epithelial cells. Kidney and plasma had levels higher than controls at all intervals.

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